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千针万线草环肽 H 的结构*

赵玉瑞[1,a] 周 俊[2,b] 王宪楷[1] 黄晓琳[3] 吴厚铭[3] 谭宁华[2] 陈昌祥[2]

([1] 华西医科大学药学院天然药物化学室, 成都 610041)

([2] 中国科学院昆明植物研究所植物化学开放研究实验室, 昆明 650204)

([3] 中国科学院上海有机化学研究所生命有机国家重点实验室, 上海 200032)

摘要　从云南民间药物千针万线草（*Stellaria yunnanensis*）的新鲜根中又得到 2 个环肽成分，其中 1 个为新环肽，命名为千针万线草环肽 H（stellarin H）。经光谱技术及化学方法证明其结构为 cyclo（Phe-Ser-Leu-Val-Leu-Pro-Pro-Tyr-Ser），另一为一已知的环肽化合物（pseudostellarin A）。

关键词　千针万线草，石竹科，环肽，千针万线草环肽 H，Pseudostellarin A

THE STRUCTURE OF STELLARIN H

ZHAO Yu-Rui[1,a], ZHOU Jun[2,b], WANG Xian-Kai[1], HUANG Xiao-Lin[3],
WU Hou-Ming[3], TAN Ning-Hua[2], CHENG Chang-Xiang[2]

([1]*Department of Medicinal Chemistry of Natural Products, School of Pharmacy, West China University of Medical Sciences*, Chengdu 610041)

([2]*Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Sciences*, Kunming 650204)

([3]*State Key Laboratory of Bio-Organic Chemistry & Natural Products, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences*, Shanghai 200032)

Abstract　In the previous papers, we have reported the isolation and structural elucidation of seven new cyclopeptides (stellarin A-G). Further chemical study on this plant resulted in the isolation of another new cyclopeptide, namely stellarin H(1). Its structure was established to be cyclo (Phe-Ser-Leu-Val-Leu-Pro-Pro-Tyr-Ser) on the basis of the spectral and chemical methods. In adition, a known cyclic pentapeptide, pseudostellarin A, was isolated.

Key words　*Stellaria yunnanensis*, Caryophllaceae, Cyclopeptides, Stellarin H, Pseudostellarin A

Considerable interest in cyclopeptides arose from their unique structures and bioactivities. As part of our ongoing investigation of bioactive cyclopeptides from plant resources, we have investigated the

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a. Department of Chemistry, Universtity of Lanzhou, Lanzhou 730000

b. Author to whom correspondence should be addressed.

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chemical constituents of the fresh roots of *Stellaria yunnanensis* and obtained seven new cyclopeptides (stellarin A—G). In the previous papers [1—4], We have reported their structure elucidation. Further chemical study on this plant led to the isolation of another new cyclopeptide named stellarin H(1) and a known cyclic pentapeptide, namely pseudostellarin A(2). In this paper, we report their isolation and structural elucidation in detail.

RESULTS AND DISCUSSION

Cyclopeptide 1, amorphous powder, $[\alpha]_D^{15}$-89.3 (c 0.033, MeOH), is negative to ninhydrin reaction but positive to the same reaction when hydrolyzed with 6 mol / L HCl. Its molecular formula was assigmed as $C_{51}H_{73}O_{12}N_9$ on the basis of FABMS [$(M+1)^+$at m / z 1004] and NMR data, indicating twenty degrees of unsaturation. The IR absorptions at $\upsilon = 3300$, 1650 cm^{-1} implied the presence of N-H and C=O groups. The ^{13}C NMR spectrum showed the presence of nine amide-carbonyls (170. 93, 171. 02, 171. 40, 172. 03, 172. 03, 172. 03, 172. 51, 173. 17 and 173. 31ppm) and nine methines (49.15, 53.79, 56.26, 56.64, 57.68, 57.79, 59.21, 60.01 and 61.56ppm) in the range of 45—65 ppm [5]. From these facts, 1 was deduced to be a peptide.

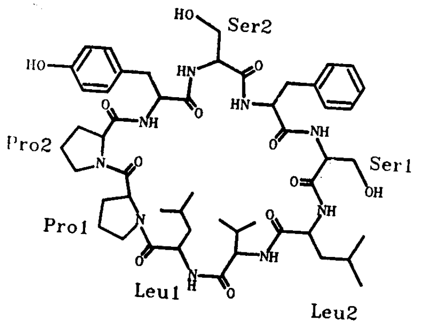

Fig.1 Stellarin H

Nine amino acid residues were identified by 2D-NMR techniques. ^1H NMR spectrum clearly exhibited the presence of seven amide protons at δ 7.860, 8.310, 8.398, 8.534, 9.177, 9.384 and 9.580 ppm. Using TOCSY and DQF-COSY spectra it was found that these amino protons constituted seven independent spin systems [4 × ABX (NH), 2 × A_3B_3MPX (NH) and 1 × A_3B_3MX (NH)]. By following the spin systems of the seven protons with use of TOCSY, DQF—COSY, HMQC and HMBC, these amino acid residues were revealed to be tyrosine, phenylalanine, valine, two serine, two leucine units. Tyrosine and phenylalanine residues all contain ABX (NH) spin systems with comparable chemical shift values and coupling constants, and therefore exhibited a similar COSY spectrum, but the differentiation between tyrosine and phenylalanine units was achieved by HMBC correlations. Utilization of 2D-NMR techniques indicated that the remaining NMR signals were found to be two indepedent spin systems of type ($X-CH-CH_2-CH_2-CH_2-X$) typical of proline. These amino acid residues accounted for mol wt. observed in FABMS. Amino acid analysis of the hydrolysate from 1 with 6 mol / L HCl by heating at 110℃ for 24h also confirmed the presence of these amino acid residues. Since only nineteen of these degrees of unsaturation could be accounted for by functionality present in the nine amino acid residues, it was apparent that 1 was a cyclic peptide.

The method of analysing peptide sequence by NOE effects is considered as a mature technique, routinely applied for peptide study [6]. For sequencing, the NOE effects between amide proton and H_α, H_β, or NH protons of the preceding amino acid residue are used. In order to determine the sepuence of the amino acid residues in stellarin H(1), a NOESY experiment was run on 1 in C_5D_5N at 300K. As shown in Fig.1, the following correlations were observed, viz, Val—H_N / Leu_2—H_α, Leu_2—H_N / Ser_1—H_α; Ser_1—

H_N / Phe—H_α; Phe—H_N / Ser—H_α, H_β; Ser_2—H_N / Tyr—H_α and Tyr—H_N / Pro_2—$H_{\delta i}$. Above results indicated the presence of the peptide fragment (—Pro_2—Tyr—Ser_2—Phe—Ser_1—Leu_2—Val—). The fragments at m / z 418, 358 observed in FABMS implied the presence of the peptide fragments (—Leu—Val—Leu—Leu—Pro, —Pro—Pro—Tyr—). The combination of these results led to the assignment of the structure of 1 as cyclo (Phe—Ser_1—Leu_2—Val—Leu_1—Pro_1—Pro_2—Tyr—Ser_2), and it was named stellarin H.

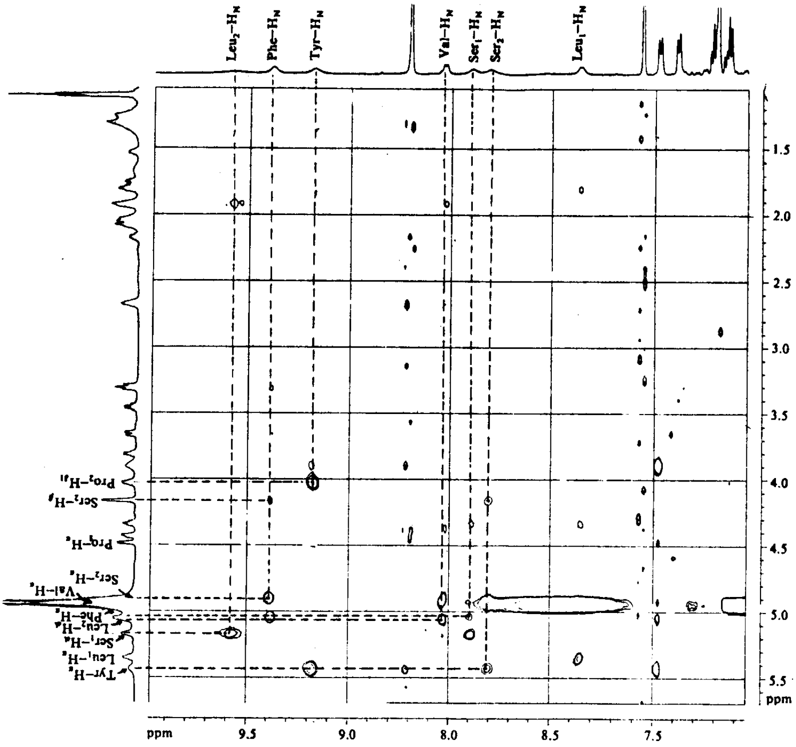

Fig.2 Lower-field part of NOESY Spectrum of 1

Cyclopeptide 2, amorphous powder, $[\alpha]_D^{17}$−100.5 (c, 0.438, MeOH), shower negative reaction to ninhydrin. Its molecular formula was determined as $C_{25}H_{35}O_6N_5$ by FABMS $[(M+1)^+$ at m / z 502] and NMR data, indicating eleven degrees of unsaturation. IR spectrum showed intense N—H and C=O absorptions at υ = 3300, 1670 cm^{-1}. The ^{13}C NMR spectrum exhibited the presence of five amide carbonyls (167.59, 170.33, 171.05 and 172.38ppm) and four methines (48.73, 53.49, 55.32 and 61.01ppm) in the range (45—65 ppm). These facts suggested that 2 is a peptide.

The extensive analyses of 2D-NMR spectra drew a conclusion that cyclopeptide 2 was composed of glycine, alanine, leucine and tyrosine and proline units. These amino acid residues accounted for mol wt. observed in FABMS. Amino acid analysis of its acid hydrolysate indicated the presence of these amino acid units established by spectroscopic methods. Since these amino acid residues accounted for only ten degrees of unsaturation, another degree of unsaturation for 2 indicated the cyclic nature of the peptide.

Sequence for amino acid residues in 2 was determined by NOESY and FABMS. The NOESY correlations (Tyr—H_N / Pro—H_α; Leu—H_N / Tyr—H_α; Ala—H_N / Leu—H_α; Gly—H_N / Ala—H_α) were observed, suggesting the presence of the peptide fragment (—Pro—Tyr—Leu—Ala—Gly—). The fragments at m / z 318, 225 observed in FABMS implied the presence of the peptide fragment (Gly—Pro—Tyr—, —Ala—Gly—Pro—). Above results led to the assigment of the structure for 2 as cyclo (Gly—Pro—Tyr—Leu—Ala), therefore it was identical with pseudostellarin A from *Pseudostellaria heterophyllia* [7].

EXPERIMENTAL

General: UV spectra were recorded on Shimadzu UV-210A in EtOH. IR spectra were taken for KBr discs with a Perkin-Elmer IR-577 infrared spectrophotometer. Optical rotations wer determined on a Jasro DIP-370 spectropolarimeter. ^1H, ^{13}C and 2D-NMR spectra were recorded with Bruker AMX-600 spectrometer, using TMS as internal standard. FABMS spectra were measured with ZAB-HS mass spectrometer.

Plant material: The roots of *Stellaria yunnanensis* Franch. were collected in Kunming, Yunnan province of China in June, 1992.

Extraction and isolation of the cyclopeptides: The fresh roots of *S. yunnanensis* (19.6 kg) were extracted three times with MeOH at reflux condition. Removal of solvents by evaporation in vacuo yielded a syrup (480 g). The syrup was suspended in H_2O, extracted with petroleum, EtOAc and n-BuOH, respectively. The EtOAc extracts were concentrated to afford a residue (37 g). The residue was subjected to column chromatography on silica gel eluting with $CHCl_3$-MeOH (5%—30%) successively to give stellarin H(19 mg, 0.00027%) and a known cyclopeptide, psudostellarin A (31 mg, 0.00047%), respectively.

Stellarin H(1), $C_{51}H_{73}O_{12}N_9$, amorphous powder, ninhydrin reaction (—). $[\alpha]_D^{17}$—89.2 (c 0.033, MeOH). UVλ_{max}^{EtOH}nm (logε): 210(3.89), 280(3.56). IRν_{max}^{KBr}cm^{-1}: 3500, 1650, 1510. FABMS m / z: 1026(M+Na)$^+$, 1004[(M+1)$^+$, base peak], 988, 907, 891, 834, 792, 766, 710, 696, 564, 544, 462, 445, 418, 375, 358, 330. ^1H NMR (600MHz, C_5D_5N, δ, ppm): Phe: 9.384(br.s, H_N), 5.054(d, 6.1, H_α), 3.653 (dd, 5.1, 13.9, $H_{\beta1}$), 3.308 (t, 10.8, $H_{\beta2}$). Tyr: 9.177 (br.s, H_N), 5.437 (br.s, H_α), 3.905 (br.s, H_β). Ser$_1$: 8.398 (br.s, H_N), 5.169 (d, 4.8, H_α), 4.423 (dd, 4.4, 9.8, $H_{\beta1}$), 4.339 (t, 9.8 $H_{\beta2}$). Ser$_2$: 8.310 (br.s, H_N), 4.853 (ca.over, H_α), 4.162 (d, 4.4, H_β). Val: 8.534 (d, 7.8, H_N), 4.955 (ca. over, H_α), 2.674 (t, 6.1, H_β), 1.094 (d, 6.8 $H_{\gamma1}$), 1.115 (d, 7.2, $H_{\gamma2}$). Leu$_1$: 7.860 (br.s, H_N), 5.355 (d, 5.9, H_α), 1.923 (ca. over, $H_{\beta1}$), 1.812 (m, $H_{\beta2}$), 2.078 (m, H_γ), 1.107 (d, 6.4, $H_{\delta1}$), 0.878(d, 6.4, $H_{\delta2}$). Leu$_2$: 9.580 (br.s, H_N), 5.055 (ca.over, H_α), 2.033(m, $H_{\beta1}$), 1.950 (ca.over, $H_{\beta2}$), 2.052 (m, H_γ), 0.824 (d, 6.2, $H_{\delta1}$), 0.726 (d, 5.6, $H_{\delta2}$). Pro$_1$: 4.486 (d, 7.7, H_α), 1.749 (m, $H_{\beta1}$), 2.664 (m, $H_{\beta2}$), 1.541 (m, $H_{\gamma1}$), 1.682 (m, $H_{\gamma2}$), 3.461 (dd, 10.8, 18.3, $H_{\delta1}$), 3.308 (t, 10.8, $H_{\delta2}$). Pro$_2$: 4.028 (d, 7.2, H_α), 2.172 (m, $H_{\beta1}$), 1.738 (m, $H_{\beta2}$), 1.923 (m, $H_{\gamma1}$), 2.042 (m, $H_{\gamma2}$), 3.819 (dd, 6.8, 15.3 $H_{\delta1}$), 4.028 (d, 7.2, $H_{\delta2}$). ^{13}C NMR (150MHz, C_5D_5N, δ, ppm): Phe: 173.17 (C=O), 56.26 (C_α), 37.63 (C_β), 138.92 (C_γ), 126.83, 128. 71, 129.48. Tyr: 173.31(C=O), 57.68(C_α), 34.12 (C_β), 128.82 (C_γ), 116.19, 131.38, 157.36. Ser$_1$: 172.51 (C=O), 56.64 (C_α), 63.02 (C_β). Ser$_2$: 172.03 (C=O), 57.79 (C_α), 61.86 (C_β). Val: 172.03 (C=O), 60.01 (C_α), 30.72 (C_β), 18.55 ($C_{\gamma1}$), 20.11 ($C_{\gamma2}$). Leu$_1$: 171.02 (C=O), 49.15 (C_α), 42.68 (C_β), 25.17 (C_γ), 22.97 ($C_{\delta1}$), 22.40 ($C_{\delta2}$). Leu$_2$: 171.40 (C=O). 53.79 (C_α), 40.86 (C_β), 25.99 (C_γ). 23.33 ($C_{\delta1}$), 23.26 ($C_{\delta2}$). Pro$_1$: 172.03 (C=O), 61.56 (C_α), 30.86 (C_β), 22.16 (C_γ), 46.86 (C_δ). Pro$_2$: 170.93 (C=O), 59.21 (C_α), 28.78 (C_β), 25.16 (C_γ), 48.18 (C_δ).

Amino acid analysis of stellarin H(1): In a routine method, amino acid analysis of the hydrolysate prepared from stellarin H(1) by heating at 110℃ for 24h in a sealed tube, showd the presence of phenylalanine (1 eq.), tyrosine (1 eq.), valine (1 eq.), proline (2 eq.), serine (2 eq.) and leucine (2 eq.)

Pseudostellarin A: amorphous powder, ninhydrin reaction (—), $[\alpha]_D^{17}$-100.5 (c 0.438, MeOH). UV λ_{max}^{EtOH}nm (logε): 204 (3.85). IR ν_{max}^{KBr}cm^{-1}: 3300, 2900, 1650, 1515, 1460. FABMS m / z: 540 $(M+K)^+$, 524 $(M+Na)^+$, 502$(M+1)^+$, 419, 391, 353, 318, 241, 225. ^{1}H NMR (600 MHz, DMSO-d_6 δppm): Gly: 7.967 (br.s, H_N), 3.939 (d, 15.5, $H_{\alpha1}$), 3.587 (d, 14.5, $H_{\alpha2}$). Ala: 8.486 (br.s, H_N), 4.143 (d, 6.9, H_α), 1.241 (d, 9.9, H_β). Leu: 8.260 (br.s, H_N), 4.008 (d, 7.9, H_α), 1.598 (dd, 6.3, 12.5, $H_{\beta1}$), 1.436 (m, 7.4, $H_{\beta2}$), 1.261 (m, H_γ), 0.901 (d, 4.8, $H_{\delta1}$), 0.798 (d, 6.5, $H_{\delta2}$). Tyr: 7.409 (d, 7.4, H_N), 4.451 (t, 7.9, H_α), 2.842 (dd, 6.5, 13.7, $H_{\beta1}$), 2.789 (dd, 9.4, 13.7, $H_{\beta2}$), 6.953 (d, 8.1, ArH), 6.629 (d, 8.1, ArH). Pro: 3.926 (d, 14.5, H_α), 1.458 (ca.over, $H_{\beta1}$), 1.979 (m, $H_{\beta2}$), 1.845 (m, H_γ), 3.914 (dd, 7.3, 10.5, $H_{\delta1}$), 3.514 (dd, 7.3, 10.5, $H_{\delta2}$), ^{13}C NMR (150 MHz, DMSO-d_6, δppm): Gly: 167.59 (C=O), 41.64 (C_α), Ala: 172.38 (C=O), 48.73 (C_α), 16.99 (C_β). Leu: 171.05 (C=0), 53.49 (C_α), 40.52 (C_β), 24.38 (C_γ), 22.48 ($C_{\delta1}$), 24.17 ($C_{\delta2}$). Tyr: 170.13 (C=O), 55.32 (C_α), 38.00 (C_β), 127.29 (C_γ), 129.86, 155.70. Pro: 170.33 (C=O), 61.01 (C_α), 29.05 (C_β), 24.17 (C_γ), 46.84 (C_δ).

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